REMARKS

Applicant acknowledges the Final Office Action concerning the U.S. Patent Application Immunodetection and Quantification Methods (Humphreys, US 2004/0137643 A1) and has filed a petition for a Request for Continued Examination (RCE). Below are additional remarks the Applicant believes are relevant to the RCE petition and respectfully requests these remarks be considered by the Examiner.

In the Final Office Action, Claim 31 stands rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. Applicant respectfully disagrees.

Claim 31 is intended to specifically claim the quantitative accuracy at low protein concentrations, as demonstrated by a low "limit of quantification," attainable through the technology described by Humphreys (see pg. 2, bottom of paragraph 0017). Applicant directs Examiner to pg.4, paragraph 0042, carried onto pg. 5:

"The signal from 6 uL aliquots of microsomes containing FLAG-C4H was measured in triplicate and quantified in comparison to this response factor (FIG. 5B). Using this method, 0.10.+-.0.009 pmol uL^{-1} FLAG-C4H was detected in microsomes of yeast carrying pYeDP60-FC4H (Table 1)."

Applicant also directs Examiner to Table 1, located on pg. 5, which presents this same data.

Although Claim 30 is not currently under examination, Applicant submits that the above information as it relates to Claim 31 is equally applicable to Claim 30.

In the Final Office Action, Claims 13, 15 and 21 stand rejected under 35 U.S.C 102(b) as being anticipated by Yabusaki et al. (US 5,436,159). Applicant respectfully disagrees.

The Examiner states (pg. 6):

"Yabusaki et al. describe a method for quantifying a P450 protein (see col. 7, line 67, '[d]etermination of expression amount)'"

The technique for P450/reductase determination (quantification) used by Yabusaki et al. is not by immunological techniques, in direct contrast to the claims of Humphreys. Instead, Yabusaki et al. describe a spectroscopic technique for quantifying Cytochrome P450s. The technique they used is carbon monoxide difference spectroscopy, which takes advantage of a spectral shift of the heme in P450s when the P450 is exposed to CO. Because Yabusaki et al. used immunological techniques to detect their P450/recuctase chimera, but quantified their P450/recuctase chimera using a spectroscopic technique, the Applicant posits that Yabusaki et al. were clearly unable to use their immunological techniques to quantify protein.

Starting at col. 8, line 57, to col. 9, line 2 Yabusaki et al. write:

"...1 ml each of the cell suspension was poured into two cuvettes, respectively and carbon monoxide was bubbled into the cuvette of sample side. Then, 5-10 mg of dithionite was added to both the cuvettes. After well stirring, difference spectrum of 400-500 nm was measured and heme-containing P-450 content was calculated based on the value Δe=91 mM^{-1} cm^{-1} from the difference in absorbance at 447 nm and 490 nm. As shown in Table 1, it was found that strains [...] produced about 6-7x10^4 molecules of heme-containing P-450/reductase chimetic fused proteins per cell, respectively."

Also concerning Yabusaki et al., the Examiner further states (pg. 6):

"contacting the P450 protein with a labeled ligand that directly binds to the epitope (see col 8, lines 28-29, "anti-reductase antibody") directly detecting the labeled ligand (see col. 8, line 36 "[^{125}I] protein A")."

The antibodies described by Yabusaki et al. are unlabeled, making the Yabusaki et al. antibody, by itself, undetectable, and making the antibody, by itself, useless for

either protein detection or quantification. This is in direct contrast to the technology described in Humphreys in which the sole antibody is itself labeled and detectable.

As described by Yabusaki et al., their antibody is contacted by a second agent, Protein A, which is a protein that nonspecifically binds antibodies. Finally, detection is accomplished by autoradiography of ^{125}I, a radioactive isotope used to label the Protein A, (which in turn binds the antibody that contacts the epitope-labeled P450). Only through the contact of a second reagent, Protien A, is the antibody (and its P450 ligand) detectable. Therefore, it is inaccurate to describe the multiple-step, multiple-reagent immunological techniques of Yabusaki et al. as "directly detecting the labeled ligand." The Applicant further suggests that the multi-step approach described by Yabusaki et al. has the potential to create significant error. In contrast, the one-step immunodetection technology described in Humphreys reduces potential error, making reliable protein quantification by western blot possible (see pg. 1, paragraphs 0002-0004).

In the Final Office Action, Claim 16 stands rejected under 35 U.S.C 103(a) as being unpatentable over Yabusaki et al. (US 5,436,159) in view of Hopp and Prickett (US 4,851,341). Applicant respectfully disagrees.

Yabusaki et al. describe a multi-step, indirect immunological technique, and utilize a spectroscopic, not immunological, technique for P450 quantification. These distinct differences between Yabusaki et al. and Humphreys severely limit the relevance of Yabusaki et al. to the claims of Humphreys, as substantially described, *supra*, and incorporated herein.

The secondary Hopp and Prickett patent fails to supplement the inadequacies of the primary Yabusaki et al. patent.

The Examiner states (pg. 7):

"Yabusaki et al. do not describe an epitope comprising the sequence Asp-Tyr-Lys-Asp-Asp-Asp-Lys (hereinafter "FLAG® tag").
However, Hopp & Prickett describe the use of FLAG® tags (see abstract) for purifying (see Title) and detecting (see col. 6, lines 27-28, "Western immunoblots") recombinant proteins.

Therefore it would have been obvious for a person of ordinary skill in the art [...] because Hopp & Prickett developed a system that 'provides superior identification and purification performance' (see col. 2, lines 56-57)..."

While Hopp and Prickett do describe the use of FLAG® tags for the immunoaffinity purification, and therefore detection, of recombinant proteins, Hopp and Prickett do not describe a method for immunologically quantifying a recombinant protein. Instead, Hopp and Prickett describe protein visualization through silver staining of SDS-PAGE gels, as well as western blotting of SDS-PAGE gels using 4E11 antibody to identify protein already purified through 4E11 antibody affinity chromatography (see col. 6, lines 26-32). The western blot was conducted to confirm "4E11-reactive material" (detection, not quantification). In direct contrast to Humphreys, no description of the use, means of antibody detection, or any suggestion of protein quantification using 4E11 antibody by western blot is described. Rather than the single-step immunological techniques described in Humphreys, Hopp and Prickett typically use gel silver staining to visualize results of 4E11 antibody affinity chromatography (see col.8, lines 26-28).

The Applicant respectfully suggests that there is a key distinction between technology useful for protein detection, and the more challenging and useful technique described in Humphreys that facilitates reliable protein quantification. This is, in part, redundant to the remarks made by the Applicant to demonstrate the inadequacies of Ro et al. in Applicant's Reply to Office action of June 3, 2005 (see pg. 6, continued to pg. 7). The emphasis on protein quantification in Humphreys is evidenced by the simple fact that the word "quantification" is used 43 times throughout the original patent application.

The Applicant notes that while the FLAG® sequence was used as an illustrative embodiment of the protein detection technique described by Humphreys, this technique is not limited to using the FLAG® sequence (see pg. 1, bottom of paragraph 0005).

In the Final Office Action, Claims 17-18 stand rejected under 35 U.S.C 103(a) as being unpatentable over Yabusaki et al. (US 5,436,159) and Hopp and Prickett (US 4,851,341), as applied to claims 13 and 15-16, and further in view of Kay (US 3,789,116). Applicant respectfully disagrees.

Yabusaki et al. describe a multi-step, indirect immunological technique, and utilize a spectroscopic, not immunological, technique for P450 quantification. These distinct differences between Yabuski et al. and Humphreys severely limit the relevance of Yabusaki et al. to the claims of Humphreys, as substantially described, *supra*, and incorporated herein.

Hopp and Prickett describe the use of FLAG® tags for the detection of recombinant proteins, but do not describe a method for quantifying a recombinant protein. This distinct difference between Hopp and Prickett and Humphreys severely limits the relevance of Hopp and Prickett to the claims of Humphreys, as substantially described, *supra*, and incorporated herein.

The tertiary Kay patent fails to supplement the inadequacies of the primary Yabusaki et al. patent and the secondary Hopp and Prickett patent.

In the Response to Arguments, the Examiner states (pg. 10):

"With respect to the teachings of Kay, Applicant argues that there is no teaching or suggestion that Kay could be used in western blots to quantitate low levels of proteins, and that persons skilled in the art of the protein sciences in 1974 would understand that Kay only provides for qualitative testing (see Applicant's reply, p. 7, first full paragraph). These arguments are not persuasive because, Examiner posits, persons skilled in the art *at the time of invention in 2002* would readily appreciate the sensitivity of fluorescence-based assays using fluorophore-conjugated antibodies, as Kay discloses such antibody reagents having 'superior sensitivity' (see col. 1, line 66) and 'excellent fluorescent characteristics including both brightness and color' (see col. 2, lines 20-24)."

First, Applicant would like to clarify the time of invention for Humphreys. The first conception, first drawing, and initial outline of Humphreys were recorded on May 22, 2000 in room B031 of the Whistler Building at Purdue University. The first test or operation with associated full written description was recorded on August 16, 2000.

Concerning Kay, the Applicant fully agrees with the Examiner that a person skilled in the art at the time of invention (be it in 2000 or 2002) would have recognized the sensitivity of fluorescence-based assays. Applicant respectfully suggests that

sensitivity, as described by Kay, is necessary, but not sufficient, for the quantification of fractions of picomoles of P450 protein. In other words, a discussion of fluorescence sensitivity is not equivalent to a discussion concerning technology developed to use this sensitivity in a way that provides quantitative information. The present application harnesses the sensitivity of fluorescence to immunologically quantify (not just detect) fractions of picomoles of a P450 protein on a western blot. In this light, the Applicant reaffirms the original points made in the Applicant's Reply to Office Action of June 3, 2005 (pg. 7):

"... While Kay does teach that antibodies can be labeled with a fluorescent chromaphore, there is no teaching or suggestion that such techniques could be used in western blot analysis to quantitate low levels of proteins. The technology described in the present application illustrates a new and novel use for chromaphore-labeled antibodies that is far beyond the scope of Kay's patent and was never envisioned by Kay. The examples provided in Kay demonstrate his vision that this technology was a qualitative (not quantitative) approach for the identification of disease-related micro-organisms and macromolecules including DNA, RNA, bacteria, fungi, protozoans, etc. (Column 1, lines 3-10 and Column 1 lines 49-63)."
"Furthermore, the limits of detection described by Kay are '500 organisms per milliliter' (Column 1, lines 64-72). In contrast, the technology described in the present application has a demonstrated limit of quantization with a minimum of a 10:1 signal to noise ratio of 0.8 picomoles (10^{-12}) moles of protein per assay."

Please note that the above "0.8 picomoles" is a typographical error originally occurring in the patent application (see pg. 6, bottom of paragraph 0052). The Applicant regrets this error. The demonstrated limit of quantification of FLAG-C4H (P450 protein) was determined to be 0.10±0.009 pmoles uL^{-1} (see pg. 5, column 1, bottom of paragraph 0042 carried over from page 4, and pg. 5, Table 1). Yeast microsome samples with a volume of six microliters were assayed, making the limit of quantification 0.6 picomoles per sample. A limit of detection was not reported.

Applicant notes that the "limit of quantification", not detection, is emphasized in Humphreys (see pg. 2, bottom of paragraph 0017). The concept of a "limit of quantification" is also used in the comparison of the techniques in Humphreys to other methods, such as CO difference spectroscopy (see pg. 5, bottom of paragraph 44 and page 5, Table 1). The Applicant suggests that convenient, reproducible, immunological protein quantification at sub-picomole levels as described by Humphreys, was not obvious to Kay in 1974, and it was not obvious to persons skilled in the art in 2000 (examples include Yabusaki et al. and Ro et al.).

In the Final Office Action, Claim 20 stands rejected under 35 U.S.C 103(a) as being unpatentable over Yabusaki et al. (US 5,436,159), Hopp and Prickett (US 4,851,341), and Kay (US 3,789,116) as applied to claims 13 and 18 in further view of Amersham Pharmacia Biotech, *What's New*, Life Science News 4 (2000). Applicant respectfully disagrees.

Yabusaki et al. describe a multi-step, indirect immunological technique, and utilize a spectroscopic, not immunological, technique for P450 quantification. These distinct differences between Yabusaki et al. and Humphreys severely limit the relevance of Yabusaki et al. to the claims of Humphreys, as substantially described, *supra*, and incorporated herein.

Hopp and Prickett describe the use of FLAG® tags for the detection of recombinant proteins, but do not describe a method for quantifying a recombinant protein. This distinct difference between Hopp and Prickett and Humphreys severely limits the relevance of Hopp and Prickett to the claims of Humphreys, as substantially described, *supra*, and incorporated herein.

Kay describes the use of fluorescent chemicals for labeling antibodies which is capable of high sensivity, but does not describe a method for quantifying a recombinant protein. This distinct difference between Kay and Humphreys severely limit the relevance of Kay to the claims of Humphreys, as substantially described, *supra*, and incorporated herein.

The quaternary Amersham advertisement fails to supplement the inadequacies of the primary Yabusaki et al. patent, the secondary Hopp and Prickett patent, and the tertiary Kay patent.

The Examiner states (pg. 9):

"However, Amersham Pharmacia Biotech teaches the use of a phosphor autoradiography imager (see Title, 'Typhoon 860 Variable Mode Imager') for scanning gels, blots and phosphor screens (see seventh bullet). Therefore, it would have been obvious for a person of ordinary shill in the art to perform the method of quantifying a P450 protein, as taught by Yabusaki et al., Hopp & Prickett, and Kay, with a phosphor autoradiography imager because Amersham Pharmacia discovered that phosphor autoradiography imagers enable 'direct chemiluminescence imaging without intermediate exposures to films or screens' (see sixth bullet)."

The Applicant notes that the instrument described in the title is a "Typhoon 8600." A key difference between what is being advertised by Amersham Pharmacia Biotech and what is described in Humphreys is found in the sixth bullet, which lists "direct chemiluminescence imaging." In contrast, the technique described in Humphreys utilizes fluorescent imaging, as discussed above (see also pg. 1, middle of paragraph 0005). Furthermore, in reference to Amersham, chemiluminescence imaging typically involves Amersham's ECL technology in which a primary antibody is followed by a secondary antibody with an attached enzyme. This enzyme runs a chemical reaction to convert a substrate molecule to a product, and in the process emits detectable light (chemiluminescence). In contrast, the present application described in Humphreys achieves quantitative accuracy at low concentrations by using a single step as described in the patent application (see p. 1, paragraphs 0003-0004). A more detailed description of the limitations of the teachings of Amersham, including the Amersham ECL technology, can be found starting at the middle of pg. 8, carried onto pg. 9, of Applicant's Reply to Office Action of June 3, 2005.

CONCLUSION

Applicant has endeavored to address all of the Examiner's concerns related to Humphreys. However, the Applicant appreciates the Examiner's thoroughness and need for caution and welcomes any suggestions by the Examiner. With this in mind, the Applicant believes Humphreys describes a truly novel technology and that the claims currently under consideration are in condition for allowance. Therefore, the Applicant respectfully requests allowance of these claims, and passage of the application to issuance.

Respectfully submitted,

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